IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Marco Ciufolini et al.

Title: 2-(3-aminoaryl)amino-4-aryl-thiazoles for the Treatment of Diseases

Appl. No.: 10/632,101

Filing Date: 08/01/2003

Examiner: Laura Stockton

Art Unit: 1626

DECLARATION UNDER RULE 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Marco A. Ciufolini, declare as follows:

1. I am one of the inventors of the captioned application.

2. My academic background and work experience are summarized in my *curriculum vitae*, which is attached as Exhibit A. Briefly, I started my independent career at Rice University, Houston, TX, in 1984, as an assistant professor of chemistry and rose to the rank of Full Professor in 1997. Being an internationally know authority in my scientific field, I was offered – and accepted – a chair of synthetic organic chemistry at the University of Lyon, France. I worked in Lyon from January 1998 until June 2004, when the University of British Columbia offered me a prestigious position as the Canada Research Chair in synthetic organic chemistry. Over the years, I have produced more than 90 research papers in synthetic organic chemistry and submitted 7 patents in the area of medicinal chemistry. I have directed the research of more than 30 Ph.D. students and 50 Master's students and founded or co-founded 3 companies (1 in the US, 2 in Europe) in which I serve, or I have served, as head of chemistry and in various other capacities. I am a consultant for several industrial laboratories in the US and Europe. My research has been recognized with various scientific awards and it

has earned me invitations to lecture at more than 180 technical meetings, industrial and academic research centers in North America, Europe and Asia.

3. I have read and understand the Office Action dated February 14, 2006 ("Office Action"). Among other rejections, I understand that the Office Action rejects the claims as obvious over the following references, either alone or in any combination:

(a) WO 00/33842 to Lago *et al.* ("Lago");

(b) U.S. Patent No. 3,467,666 to Dexter *et al.* ("Dexter");

(c) U.S. Patent No. 3,201,409 to Spivack *et al.* ("Spivack");

(d) U.S. Patent No. 6,291,514 to Illig *et al.* ("Illig");

(e) U.S. Pat. Appl. Pub. No. 2003/0158199 to Steiber *et al.* ("Steiber"); and

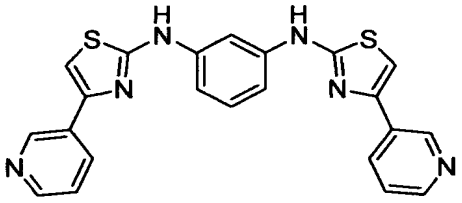
(f) U.S. Pat. Appl. Pub. No. 2001/0044545 to Dhanoa *et al.* ("Dhanoa").

The Office Action states that references (a)-(f) "each teach substituted phenylamino-2-thiazole compounds that are structurally similar to the instant claimed compounds." Office Action at 11. The Office Action concludes that one of skill in the art would be motivated to make the specifically claimed compounds "from the expectation that structurally similar compounds would possess similar activity (e.g., antagonizing the myt1 kinase receptor)." *Id.* at 12.

4. I have directed experiments comparing the activity of the claimed compounds to the compounds disclosed by references (a)-(f). The activity of a compound is measured by its ability to inhibit cell proliferation and is expressed as a percentage of cell proliferation obtained in the absence of treatment (%proliferation). CPM (counts per minute) represent the radiolabed thymidine incorporated by proliferating cells and the IC50 defines the concentration of compound necessary to obtain 50% inhibition of proliferation of a target cell. Specifically, the experiments investigated the inhibitory effects, or lack thereof, that the tested compounds exhibited on different tyrosine kinases, including wild-type (WT) c-kit and mutant forms of c-kit. These experiments and their significance are summarized below.

Activity of Cited Compounds on Tyrosine Kinases

5. In one experiment, we tested the effects of the following compound from Lago:



1512

Lago at page 3, line 17. Lago generally relates to structures for inhibiting MYT1 kinase, which belongs to the Casein Kinase 1 family. MYT1 kinase has a very different structure and properties compared to the stem-cell factor receptors, such as c-kit. Nevertheless, we tested the compound of Lago on a variety of tyrosine kinases, including several forms of c-kit. The results of the experiments are shown in Fig. 1.

6. Table 1 shows the percent activity of different tyrosine kinases tested as a function of the concentration of Lago's compound (1512). Specifically, the following tyrosine kinases were tested:

(a) Ba/F3 is a murine hematopoietic cell line cultured in presence of IL-3. IL-3 is necessary for the survival and growth of Ba/F3 cells. IL-3 allows to rescue the cells when there are in contact with a potent and non toxic inhibitor. On the contrary, when cells die the inhibitor is considered toxic and unsuitable for therapy : (Ba/F3 +IL3)

(b) Ba/F3 expressing human c-kit receptor (wild type) cultured in presence of IL-3. IL-3 is necessary for the survival and growth of Ba/F3 cells. IL-3 allows to rescue the cells when there are in contact with a potent and non toxic c-kit inhibitor. On the contrary, when cells die the inhibitor is considered toxic and unsuitable for therapy : (Ba/F3 hKIT WT+ IL3)

(c) Ba/F3 expressing human c-kit receptor tyrosine kinase (wild type) cultured in presence of its ligand : SCF. SCF activates c-kit which in turns promotes cell proliferation. Inhibition of proliferation in presence of SCF means that the inhibitor is specific to c-kit since the same inhibitor does not inhibit the same

cell line in the presence of IL3 (see paragraph b above). (Ba/F3 hKIT WT +SCF).

(d) Ba/F3 expressing murine or human c-kit (mutated in the enzymatic region [TK] at codon 814 and 816 respectively). Such mutation has been found in Acute Myeloid Leukaemia, Germ Cell Tumors and Mastocytosis. TK mutation constitutively activates c-kit which in turns promotes cell proliferation. Inhibition of proliferation in presence of SCF means that the inhibitor is specific to mutated TK c-kit. (Ba/F3 mKIT D814V and Ba/F3 hKIT D816V)

(e) Ba/F3 expressing murine c-kit (mutated in the juxtamembrane region [JM]) . JM region in c-kit has been shown to be a negative regulatory region for c-kit activation. Such mutation has been found in GIST (gastro Intestinal Stromal Tumors). JM mutation constitutively activates c-kit which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to mutated JM c-kit. (Ba/F3 mKIT delta27)

(f) Ba/F3 expressing Tel-Jak1. Jak1 is a cytoplasmic kinase from the same family as Jak2. Tel-Jak1 is a genetically enginered gene that is the result of the fusion between the Jak1 tyrosine kinase gene and the TEL (ETV6) gene. Tel-Jak1 fusion mutation results in constitutive Jak1 tyrosine kinase activity and has been shown to have oncogenic properties and promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to Jak1 kinase. (Ba/F3 TEL-JAK1)

(g) Ba/F3 expressing Tel-Jak2. Tel-Jak2 is a genetic alteration that is the result of the fusion between the Jak2 tyrosine kinase gene and the TEL (ETV6) gene. This fusion has been identified in cases of human childhood T-cell acute lymphoblastic leukemia (ALL), pre-B cell and atypical chronic myeloïd leukemia (CML). Tel-Jak2 fusion mutation results in constitutive Jak2 tyrosine kinase activity and has been shown to have oncogenic properties and promotes

cell proliferation. Inhibition of proliferation means that the inhibitor is specific to Jak2 kinase. (Ba/F3 TEL-JAK2)

(h) Ba/F3 expressing Tel-Jak3. Jak3 is a cytoplasmic tyrosine kinase from the same family as Jak2.Idem as described for Jak1above. (Ba/F3 TEL-JAK3)

(i) Ba/F3 expressing Tel-Tyk2. Tyk2 is a cytoplasmic tyrosine kinase from the same family as Jak2. Idem as described for Jak1above. (Ba/F3 TEL-TYK)

(j) Ba/F3 expressing H4-Ret. H4-Ret is a genetic alteration that is the result of the fusion between the Ret tyrosine kinase region of the receptor gene and the 55 KDa nuclear and cytosolic protein encoded by H4 gene. This fusion has been identified in cases of human thyroid papillary carcinomas. H4-Ret fusion results in constitutive Ret tyrosine kinase activity, It has been shown to have oncogenic properties and promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to Ret kinase. (Ba/F3 H4 RET)

(k) Ba/F3 expressing human Bcr-Abl. Bcr-Abl protein results from a reciprocal translocation between the breakpoint cluster region (bcr) and the tyrosine kinase domain of Abl kinase. This fusion has been identified in cases of Chronic Myeloid Leukaemia. Bcr proteins promote constitutive dimerisation of the Bcr-Abl chimeric proteins and lead to constitutive activity of Abl kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to Abl kinase. (Ba/F3 BCR-ABL)

(l) Ba/F3 expressing human FLT3 receptor tyrosine kinase (wild type) cultured in presence of its ligand : FL. FL activates FLT3 which in turns promotes cell proliferation. Inhibition of proliferation in presence of FL means that the inhibitor is specific to FLT3. (Ba/F3 hFLT3 WT +FL)

(m) Ba/F3 expressing human FLT3 receptor tyrosine kinase (mutated by a internal tandem duplication of the sequence in the juxtamembrane region [JM ITD]). JM region in FLT3 has been shown to be a negative regulatory region for

FLT3 activation. Such mutation has been found in Acute Myeloid Leukaemia. They constitutively activate FLT3 kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to mutated JM FLT3. (Ba/F3 hFLT3 ITD)

(n) Ba/F3 expressing EGFR-FGFR1. EGFR-FGFR1 is a genetically engineered chimeric protein between the extracellular region of the EGFR and the tyrosine kinase domain of FGFR1 tyrosine kinase receptor. Addition of EGF ligand leads to dimerisation of the ligand binding domain of EGFR that lead to constitutive activity of FGFR1 kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to FGFR1 kinase. (Ba/F3 EGFR-FGFR1 +EGF)

(o) Ba/F3 expressing EGFR-FGFR3. EGFR-FGFR3 is a genetically engineered chimeric protein between the extracellular region of the EGFR and the tyrosine kinase domain of FGFR3 tyrosine kinase receptor. Addition of EGF ligand leads to dimerisation of the ligand binding domain of EGFR that lead to constitutive activity of FGFR3 kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to FGFR3 kinase. (Ba/F3 EGFR-FGFR3 +EGF)

(p) Ba/F3 expressing EGFR-PDGFR. EGFR-PDGFR is a genetically engineered chimeric protein between the extracellular region of the EGFR and the tyrosine kinase domain of PDGFRβ tyrosine kinase receptor. Addition of EGF ligand leads to dimerisation of the ligand binding domain of EGFR that lead to constitutive activity of PDGFRβ kinase which in turns promotes cell proliferation. Inhibition of proliferation means that the inhibitor is specific to PDGFRβ kinase. (Ba/F3 EGFR-PDGFR +EGF)

(q) Ba/F3 expressing EGFR. EGFR is a tyrosine kinase receptor. Addition of EGF ligand leads to dimerisation of the ligand binding domain of EGFR that lead to activation of the kinase which in turns promotes cell proliferation. Inhibition of

proliferation means that the inhibitor is specific to EGFR kinase. (Ba/F3 EGFR +EGF)

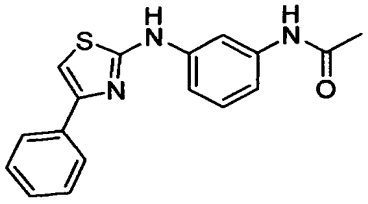
7. The results show that Lago's compound has some activity on wild-type c-kit. However, this is purely accidental, and I do not see any motivation to start from Lago's compounds, including the compound tested, to arrive at the claimed compounds that are selective c-kit inhibitors. Indeed, Lago's compounds are disclosed as inhibiting an entirely different type of kinase, MYT1 kinase, compared to the claimed compounds, which inhibit c-kit.

8. In addition, the results shown in Figure 1 and Table 1 demonstrate that the Lago compound is not a selective c-kit inhibitor. Specifically, Lago's compound does not discriminate between mutated c-kit and wild type c-kit nor does it discriminate between different tyrosine kinases. As shown in Figure 1 and Table 1, the Lago compound has inhibitory activity on all the tyrosine kinases tested including Receptor Tyrosine Kinase (RTK) and non-RTK.

9. These data demonstrate that Lago's compound exhibits a non-specific inhibitory activity towards a large number of tyrosine kinases, including RTK and non-RTK. This lack of specificity may translate into general cell cytotoxicity making these compounds poorly suited, or entirely unsuitable, for therapeutic use.

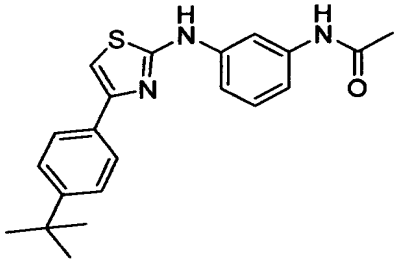
10. We also tested the effects of other compounds from the cited references. The compounds tested are shown below:

Dexter compound of example 13 (col. 9, ll. 25-43):



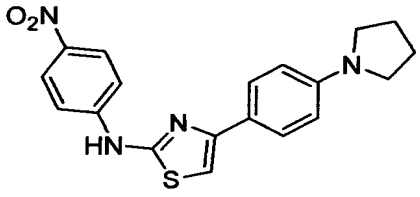
1514

Spivack compound of example 19 (col. 9, ll. 59-64):



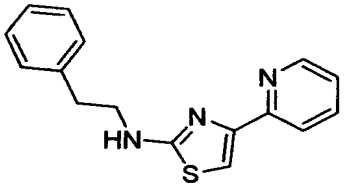
1515

Stieber compound 17 (page 7):



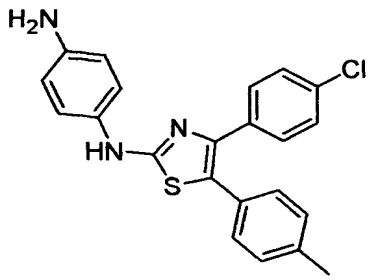
1511

Stieber compound 35 (page 10):



1516

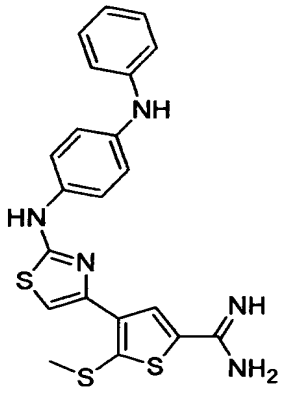
Dhanoa compound 24 (page 6, ¶ [0120]):



1529

11. None of these compounds showed any activity on any of the c-kits tested except for the Spivack compound (1515). The Spivack compounds exhibit some slight activity on one of the c-kit mutants, the c-kit delta27 mutant, at IC50=7.5μM (Fig. 3). On the other hand, the claimed compounds showed an inhibitory activity against delta27 mutant at much lower concentration (IC50=0.1μM or less) (see the biological results in the figures enclosed).

12. A compound from Illig was not tested. The Office Action cited the following compound from Illig (col. 18, ll. 25-26):

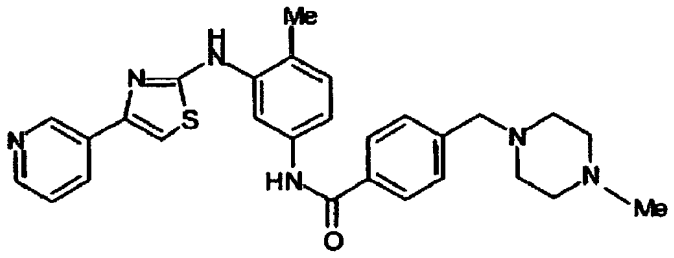


However, this compound is very difficult to synthesize and would require at least fourteen synthesis steps. In light of this complicated synthesis and the teachings of Illig, we decided not to test this compound. Indeed, the compound is a 1,4-diamino derivative and also an amidine derivative known to be protease inhibitor, a completely different therapeutic class of kinase

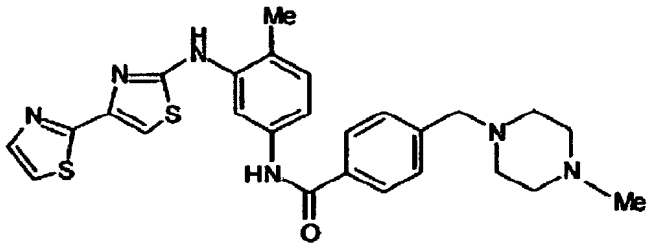
inhibitors. Thus, we did not expect this compound to have any activity on c-kit. In addition , Illig does not provide any suggestion to modify its compounds or the compounds of the other cited references to arrive at the claimed compounds.

Activity of Claimed Compounds on Tyrosine Kinases

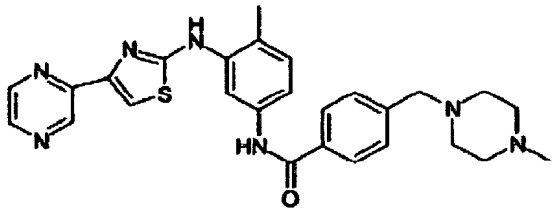
13. To compare the effects of the claimed compounds to those of Lago and the other cited references, we duplicated the above experiments using claimed compounds rather than compounds from the cited references. Specifically, we tested the following compounds from the present specification:



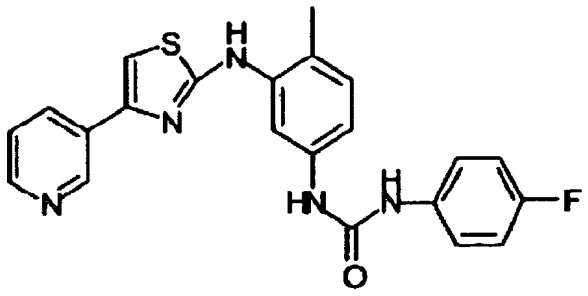
002 (pg. 20)



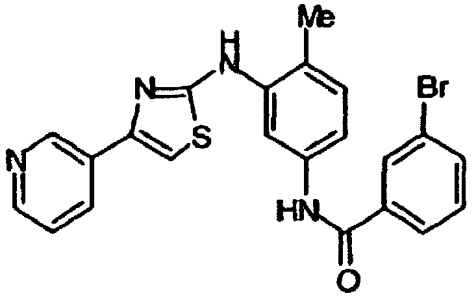
005 (pg. 21)



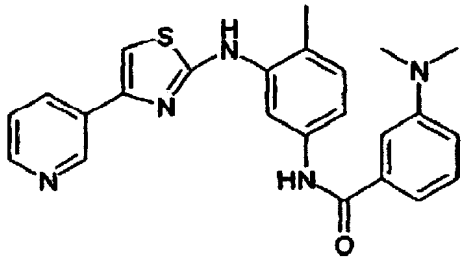
006 (pg. 21)



012 (pg. 30)



028 (pg. 40)



and 088 (pg. 73).

The results of these tests are show in Figures 7 and 8 and summurized in the following table:

Table 1: Comparing cited compounds (*) and claimed compounds (°): activities are expressed in % inhibition of cell proliferation at 1μM

Compounds / Targets	*1512** *Lago*	*1514** *Dexter*	*1515** *Spivack*	*1511** *Stieber*	*1516** *Stieber*	*1529** *Dhanoa*	*002°*	*005°*	*006°*	*012°*	*028°*	*088°*
Ba/F3 hKit +IL3	60	15	0	10	0	0	20	20	0	0	40	50
Ba/F3 m/hKit +SCF	60	15	0	10	0	0	80	50	0	100	100	100
Ba/F3 mKit D814 / hKitD816	60	15	0	10	0	0	0	0	0	0	40	75
Ba/F3 mKit Delta27	60	15	0	10	0	0	100	100	100	100	100	100
Ba/F3 TEL-JAK1	60						*0*	*0*	*ND*	*ND*	*0*	*0*
Ba/F3 TEL-JAK2	60						*0*	*20*	*0*	*0*	*10*	*0*
Ba/F3 TEL-JAK3	60						*0*	*0*	*ND*	*0*	*0*	*0*
Ba/F3 TEL-TYK	60						*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
Ba/F3 H4-RET	60						*ND*	*0*	*0*	*0*	*0*	*0*
Ba/F3 BCR-ABL	60						20	20	0	0	0	50
Ba/F3 hFLT3WT	60						*0*	*0*	*0*	*0*	*40*	*0*
Ba/F3 hFLT3ITD	60						*0*	*0*	*0*	*0*	*40*	*0*
Ba/F3 EGFR-FGFR1	60						*0*	*0*	*0*	*0*	*0*	*0*
Ba/F3 EGFR-FGFR3	60						*40*	*0*	*10*	*0*	*0*	*35*
Ba/F3 EGFR-PDGFR	60						*100*	*100*	*100*	*30*	*90*	*80*
Ba/F3 EGFR	60						*40*	*0*	*0*	*0*	*0*	*0*

ND=Not Determined

14. The claimed compounds demonstrated a high specificity to wild-type c-kit and/or mutants as well as to the structurally related PDGFRβ, unlike the compounds of the cited references. For example, compounds 005 and 006 from the present specification are more selective to the c-kit Del27 mutated in the Juxtamembrane (JM) Domain (Figure 7 and Table 1), while compounds 028 and 088 are selective to both c-kit Del27 and c-kit WT but not to c-kit D816 mutant if compared with the toxicity control Ba/F3 cKIT + IL3 (Figure 8 and Table 1). Compounds 002 and 012 are selective to both c-kit Del27 and c-kit WT with a preference for mutated c-Kit Del27 (Figures 7, 8 and Table 1). None of these compounds, nor any other claimed compounds tested, display an equal activity on the different forms of c-kit. In other words, they are selective. In addition, neither exhibits significant inhibitory activity on the non-RTK Jak2 (+IL3) and BCR-ABL kinases unlike the results seen for the Lago compound.

15. This high degree of specificity has important practical applications. For example, the specificity allows the claimed compounds to be used to treat diseases characterized in expression of c-kit, whether wild-type or a mutant form. For example, the claimed compounds could be used in the treatment of mast cell-dependent diseases in which mast cells abnormally proliferate upon activation of c-kit, including inflammatory or auto-immune diseases, as well as in certain type of tumour associated with the expression of activated JM c-Kit mutants, such as Gastrointestinal Stromal Tumour ("GIST").

16. One of the claimed compounds, AB1010, has already been granted orphan drug status by the U.S. Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA) for the treatment of a number of human malignancies for which tyrosine kinase c-kit has been shown to play a key role when activated.

These malignancies include GIST, seminoma, mastocytosis, NK cell lymphoma, acute myelogenous leukemia, small-cell lung cancer, and ovarian cancer. In addition, it has been granted orphan drug status for the treatment of mast cell-dependent disorders including inflammatory and autoimmune diseases.

Conclusion

17. The claimed compounds are unique in their properties and differ from the cited references in ways that were not expected. For example, the claims compounds demonstrated a high specificity to wild-type c-kit and/or mutants. On the other hand, nearly all of the compounds cited in the Office Action fail to demonstrate any significant inhibitory activity on c-kit. Of the compounds that did exhibit some activity on c-kit, the activity was non-specific. None of the cited references provides any motivation to alter their compounds to arrive at the claimed compounds. Indeed, the cited references do not relate to inhibition of C-KIT.

* * * * *

18. I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Jan 22, 2007
Date:

Marco A. Ciufolini